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Isolation of *Arthrobacter* spp. from Clinical Specimens and Description of *Arthrobacter cumminsii* sp. nov. and *Arthrobacter woluwensis* sp. nov.

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Arthrobacter **spp. are very widely distributed in the environment (e.g., soil) but have not been described as causing disease in humans. Over a 6-year period, two reference laboratories isolated or received 11 strains which were eventually identified as belonging to the genus *Arthrobacter*. These strains had been initially identified as Centers for Disease Control and Prevention coryneform group B-1 and B-3 bacteria (whitish-grayish colonies of 2 mm or greater in diameter after 24 h of incubation, respiratory metabolism, absent or weak acid production from sugars, and hydrolysis of gelatin). However, chemotaxonomic investigations revealed lysine as the diamino acid of the cell wall and the presence of branched cellular fatty acids (with *anteiso*-pentadecanoic acid predominating) which was compatible with an assignment of the 11 isolates to the genus *Arthrobacter* only. Peptidoglycan and 16S rRNA gene sequence analyses demonstrated that three of the strains studied were representatives of a new *Arthrobacter* species for which the name *Arthrobacter cumminsii* sp. nov. is proposed and that one other strain represented a second new *Arthrobacter* species for which the name *Arthrobacter woluwensis* sp. nov. is proposed. This report is the first on the isolation of *Arthrobacter* spp. from clinical specimens.**

It is only in recent years that clinical microbiologists have begun to fully recognize the enormous diversity of coryneform bacteria encountered in clinical specimens (7). In addition to many newly defined species and genera, some well-established taxa (e.g., *Aureobacterium* spp., *Microbacterium* spp., and *Cellulomonas* spp.), which by the early 1990s were better known to environmental than to clinical microbiologists, have been demonstrated to be clinically relevant (7). In an attempt to identify to the species level some clinically significant strains corresponding to Centers for Disease Control and Prevention (CDC) coryneform group B-1 and B-3 bacteria (9) which were isolated by or referred to two reference laboratories in Europe and North America and which were not *Brevibacterium* spp. (5, 8), we observed that these isolates are members of the genus *Arthrobacter*. To the best of our knowledge, strains belonging to the genus *Arthrobacter* have not so far been described as being isolated from clinical specimens. *Arthrobacter* strains are widely distributed in the environment, sometimes representing the most numerous single bacterial group in aerobic plate counts of specimens from soil (12). They exhibit considerable nutritional versatility (except *Arthrobacter citreus*) with a wide range of organic compounds (12), and so far, it has almost been impossible to differentiate the 17 currently defined *Arthrobacter* species by phenotypic characters (13–15). The yellow-pigmented *Arthrobacter citreus* (13), the red-pigmented *Arthrobacter agilis* (14), *Arthrobacter ilicis* (2), and *Arthrobacter sulfureus* (24) were not included in the present comparative phenotypic analysis because they do not grow at 37°C, which would inhibit their detection in the routine processing of clinical specimens. By using 16S rRNA gene sequence and peptidoglycan analyses, we demonstrated that some of the strains studied were unambiguously representatives of two new *Arthrobacter* species for which the names *Arthrobacter cumminsii* sp. nov. and *Arthrobacter woluwensis* sp. nov. are proposed.

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MATERIALS AND METHODS

Strains, media, and growth conditions. The reference strains used in the study are listed in Table 1. Some clinical strains were isolated by workers in the Department of Medical Microbiology at the University of Zürich (DMMZ) by established methods (4) or were referred to it for identification. The remaining clinical strains were referred to the Special Bacteriology Laboratory at the Laboratory Centre for Disease Control (LCDC), Ottawa, Ontario, Canada, for identification. All strains were cultured on Columbia agar (Difco, Detroit, Mich.) with 5% sheep blood (SBA) at 37°C in a 5% CO_2 atmosphere. The CAMP reaction was performed with *Staphylococcus aureus* ATCC 25923.

Biochemical tests. Preparation of the traditional media used for biochemical characterization of the strains studied was done as described by Nash and Krenz (16). All biochemical tests were performed at 37°C. Motility was observed by the hanging drop method by incubating cells in Trypticase soy broth (all media were from Becton Dickinson, Cockeysville, Md., unless stated otherwise). Nitrate reduction was tested in nitrate broth (Difco), esculin hydrolysis was observed on modified esculin agar, and hydrolysis of urea was observed in Christensen's urea broth (16). Acid production from glucose, maltose, sucrose, mannitol, and xylose was observed in cystine Trypticase agar (CTA) medium containing 1% of the carbohydrates (Sigma Chemical Co., St. Louis, Mo.). The type of metabolism was also observed by using CTA medium, with acid production or alkalinization at the surface and no change at the bottom of the tube indicating an oxidative metabolism. Assimilation reactions were tested in the system outlined by Funke and Carlotti (5) (i.e., applying the AUX medium [API bioMérieux, Marcy l'Etoile, France] in the API CH50 system [API bioMérieux]), and the results were read after 48 h of incubation. Enzymatic activities were determined by means of the API Zym system (API bioMérieux) by following the instructions given by the manufacturer. DNase production was tested with DNase test agar with methyl green (Difco). Gelatin hydrolysis was detected by immersing film strips (Diagnostics Pasteur, Marnes-la-Coquette, France).

Antimicrobial susceptibility patterns. Susceptibility to antimicrobial agents

TABLE 1. Strains used in the present study

Organism	Strain no.[a]	Source
Reference strains (all type strains)		
Arthrobacter globiformis	DSM 20124 (ATCC 8010)	NK[b]
Arthrobacter atrocyaneus	CIP 102365 (ATCC 13752)	Air
Arthrobacter aurescens	ATCC 13344	Soil
Arthrobacter crystallopoietes	CIP 102717 (ATCC 15481)	Soil
Arthrobacter histidinolovorans	ATCC 11442	Soil
Arthrobacter nicotinovorans	DSM 420	NK
Arthrobacter oxydans	DSM 20119 (ATCC 14358)	Air
Arthrobacter pascens	ATCC 13346	Soil
Arthrobacter ramosus	ATCC 13727	Soil
Arthrobacter ureafaciens	ATCC 7562	Soil
Arthrobacter nicotianae	CIP 82.107 (ATCC 15236)	Air
Arthrobacter protophormiae	ATCC 19271	*Protophormia terraenovae*
Arthrobacter uratoxydans	ATCC 21749	Humus soil
Clinical strains		
Arthrobacter cumminsii	DMMZ 445 (DSM 10493^T)[c]	Urine
Arthrobacter cumminsii	DMMZ 483 (DSM 10494)	Urine
Arthrobacter cumminsii	DMMZ 537	Skin infection
Arthrobacter woluwensis	CUL 1808 (DSM 10495^T)	Blood culture
Arthrobacter sp.	DMMZ 1369	Vaginitis
Arthrobacter sp.	LCDC 90-0364	Endophthalmitis
Arthrobacter sp.	LCDC 91-0435	Endophthalmitis
Arthrobacter sp.	LCDC 92-0385	Blood culture
Arthrobacter sp.	LCDC 92-0394	Blood culture
Arthrobacter sp.	LCDC 92-0600	Blood culture
Arthrobacter sp.	LCDC 93-0702	Unknown (clinical)

[a] DSM, German Collection of Microorganisms; ATCC, American Type Culture Collection; CIP, Culture Collection Institute Pasteur; DMMZ, Department of Medical Microbiology, University of Zürich; CUL, Culture Collection Catholic University of Louvain; LCDC, Laboratory Centre for Disease Control.
[b] NK, not known.

used in the treatment of infections caused by coryneform bacteria was determined by following the guidelines for performance and interpretation of the National Committee for Clinical Laboratory Standards (NCCLS) (17–19). MICs were determined by the agar dilution procedure (Mueller-Hinton agar supplemented with 5% sheep blood). Interpretation of the MICs of amoxicillin-clavulanic acid, ampicillin, and penicillin was according to the NCCLS categories for staphylococci.

Chemotaxonomic investigations. For analysis of the cellular fatty acid (CFA) patterns, cells were processed as described previously (26), and gas-liquid chromatography was performed on the Sherlock system (Microbial ID, Inc., Newark, Del.). The diamino acid of the total cell walls was determined by the method of Schaal (20). Analysis of partial peptidoglycan structures was performed by following the methods given by Schleifer and Kandler (21, 22), except that ascending thin-layer chromatography on cellulose sheets (Merck, Darmstadt, Germany) was used. The method for the determination of the G+C content of the bacterial DNA was given in an earlier report (6).

16S rRNA gene sequence analyses. 16S rRNA genes were amplified by PCR with universal primers pA and pH* (10) and were sequenced as described previously (6). The determined sequences and those of reference organisms obtained from the EMBL Data Library were aligned by using the program PILEUP (3). A distance matrix was produced with the program DNADIST of the PHYLIP package (3), and a tree was constructed by the neighbor-joining method with the program NEIGHBOUR of the same package. The stability of groupings was assessed by bootstrapping by using the programs SEQBOOT, DNADIST, NEIGHBOUR, and CONSENSE (3). Two hundred replicates were performed.

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S rRNA genes of strains DMMZ 445 and CUL 1808 have been deposited in the EMBL Data Library under the accession numbers X93354 and X93353, respectively.

RESULTS

The sources of the 11 clinical strains included in the study are listed in Table 1. Strains DMMZ 445, DMMZ 483, and DMMZ 537 were isolated in significant numbers ($>10^5$ CFU/ml) and grew in pure culture. Strain CUL 1808 was isolated from a total of five cultures of blood taken at three different times during a 4-day period from a 33-year-old female originating from Zaire who suffered from human immunodeficiency virus infection, stage C-3 (CDC criteria). She was hospitalized with a temperature of 39°C and chills. Clinical examination revealed an infection at the site of an implanted Port-a-Cath which was used for long-term treatment of cytomegalovirus retinitis. The patient improved by treatment with intravenous ampicillin and was discharged after 2 weeks. No material was cultured from the Port-a-Cath, nor could the device be removed for culturing. We were not able to evaluate the clinical significance of the other strains included in the study.

The colonies of all reference and clinical strains were whitish-grayish, 2 mm or greater in diameter, convex, and slightly glistening after 24 h of incubation on SBA. Gram stains showed coryneform bacteria of different lengths (1 to 4 μm) after 24 h and mainly coccoid cells after 72 h of incubation. Joint-like structures (i.e., cells arranged at an angle to give L or V formations) were only occasionally observed after 24 h of incubation. None of the strains examined grew under anaerobic conditions. All strains were catalase positive and showed an oxidative metabolism. None of the strains studied exhibited a positive CAMP reaction. All strains showed DNase activity and hydrolysis of gelatin within 10 days. The reactions for nitrate, esculin, and urea are given in Table 2. Reactions for utilization of 49 carbohydrates were tested for all strains, but only the 10 substrates with the greatest potential for differentiation are given in Table 2. In line with the results of Keddie et al. (13), we observed that most of the *Arthrobacter* strains tested were able to utilize the following substrates: glycerol, D-xylose, galactose, D-glucose, D-fructose, D-mannose, mannitol, salicin, cellobiose, maltose, sucrose, trehalose, melezitose, and raffinose. When applying the scheme given in Table 2, strains DMMZ 445, DMMZ 483, and DMMZ 537 as well as strain CUL 1808 could be separated from all other defined

TABLE 2. Biochemical characteristics of the strains studied[a]

Strain	Nitrate reduction	Esculin hydrolysis	Assimilation of[b]:										Other traits[c]
			ERT	βMX	RHA	DUL	INO	SOR	LAC	XYL	DAR	5KG	
A. globiformis	−	−	−	−	+	+	+	+	−	(+)[d]	−	+	
A. atrocyaneus	+	(+)	−	+	(+)	−	+	−	−	−	−	−	Urea+
A. aurescens	−	+	−	+	(+)	−	−	−	+	−	−	+	
A. crystallopoietes	+	−	−	−	−	−	+	−	−	−	−	−	Urea+
A. histidinolovorans	−	+	−	+	−	−	+	−	−	−	−	−	
A. nicotinovorans	−	−	−	+	+	−	+	−	−	−	−	(+)	
A. oxydans	+	−	−	+	−	−	+	+	+	+	−	−	GLU+, SUC+
A. pascens	−	−	+	−	+	−	+	+	−	−	−	(+)	SUC(+)
A. ramosus	−	−	+	−	+	−	+	+	−	−	−	+	
A. ureafaciens	−	+	−	+	(+)	−	+	−	−	−	−	−	
A. nicotianae	+	−	−	−	−	−	−	−	−	−	+	−	
A. protophormiae	+	−	−	−	−	−	−	−	−	−	−	+	
A. uratoxydans	+	−	−	−	−	−	−	−	−	−	−	−	Urea+
A. cumminsii DMMZ 445[e]	−	−	−	−	−	−	−	−	−	−	−	−	
A. cumminsii DMMZ 483[e]	−	−	−	−	−	−	−	−	−	−	−	−	
A. cumminsii DMMZ 537[e]	−	−	−	−	−	−	−	−	−	−	−	−	
A. woluwensis CUL 1808	−	+	−	−	−	+	−	+	+	−	+	+	Urea(+)
Arthrobacter sp. strain DMMZ 1369	−	−	−	+	−	−	+	+	+	+	−	−	SUC(+)
Arthrobacter sp. strain LCDC 90-0364	−	+	−	(+)	+	−	−	+	−	(+)	−	(+)	
Arthrobacter sp. strain LCDC 91-0435	+	−	−	+	(+)	−	+	−	−	−	−	−	Urea(+), GLU(+), MAL(+)
Arthrobacter sp. strain LCDC 92-0385	+	+	−	+	(+)	−	+	+	−	+	−	+	
Arthrobacter sp. strain LCDC 92-0394	−	+	−	−	+	−	−	−	−	−	−	−	
Arthrobacter sp. strain LCDC 92-0600	−	+	−	−	+	−	−	−	−	−	−	−	
Arthrobacter sp. strain LCDC 93-0702	+	−	−	(+)	−	−	+	+	+	(+)	−	−	GLU(+), SUC+

[a] All strains with oxidative metabolism; no acid production from glucose, maltose, sucrose, mannitol, or xylose except for those mentioned; urease negative except for those noted.

[b] ERT, erythritol; βMX, β-methyl-xyloside; RHA, rhamnose; DUL, dulcitol; INO, inositol; SOR, sorbitol; LAC, lactose; XYL, xylitol; DAR, D-arabitol; 5KG, 5-keto-gluconate.

[c] GLU+, MAL+, SUC+, oxidative acid production from glucose, maltose, or sucrose, respectively.

[d] Parentheses indicate a weak reaction.

[e] *A. cumminsii* strains did not show any utilization reaction in the system used.

Arthrobacter species. The same was true for most of the LCDC strains with the exception of strain LCDC 91-0435, which exhibited a profile similar to those of *Arthrobacter ureafaciens* and *Arthrobacter atrocyaneus*. Strains LCDC 93-0702 and DMMZ 1369 showed patterns similar to those of *Arthrobacter oxydans*. In addition, strains LCDC 92-0394 and LCDC 92-0600 exhibited identical patterns. *Arthrobacter pascens* and *Arthrobacter ramosus* showed closely related biochemical profiles.

Table 3 gives the antimicrobial susceptibility patterns of all strains studied. Penicillins showed markedly better activity than the cephalosporins, narrow-spectrum cephalosporins usually had lower MICs than expanded- or broad-spectrum cephalosporins, and penicillin exhibited lower MICs than imipenem. All strains were susceptible to teicoplanin and vancomycin. Tetracycline MICs were lower than the NCCLS breakpoint for susceptibility for all strains except DMMZ 483 and DMMZ 537. Strain LCDC 93-0702 was the only strain resistant to chloramphenicol, whereas the *A. ureafaciens* and *Arthrobacter uratoxydans* strains showed in vitro resistance to rifampin. Ciprofloxacin, clindamycin, and gentamicin had only limited activity against the strains examined. Strain CUL 1808 was multiresistant, being susceptible to tetracycline, teicoplanin, and vancomycin only.

anteiso-Pentadecanoic acid ($C_{15:0ai}$) was the predominant CFA in all strains examined (Table 4). Significant amounts of $C_{15:0i}$, $C_{16:0i}$, $C_{16:0}$, and $C_{17:0ai}$ were also detected, depending on the individual strains. No species-specific CFA patterns were found. CFA profiles were qualitatively similar for all strains examined and suggested an assignment of the clinical strains to the genus *Arthrobacter* when the profiles were compared with those of the reference strains. Thin-layer chromatography of whole-cell hydrolysates of the clinical strains revealed lysine as the diamino acid of the cell wall, consistent with the genus *Arthrobacter* (13). The detailed structures of the purified peptidoglycans from strains DMMZ 445 and CUL 1808 were also elucidated. Fragments of the primary structure of the peptidoglycans of these strains are illustrated in Fig. 1. Strain DMMZ 445 possessed an A4α, L-lysine (LYS)–L-serine (SER)–L-glutamic acid (GLU)-type murein. A small amount of the serine residue (ca. 20%) was found to be replaced by glycine (GLY) in this strain. By contrast, strain CUL 1808 possessed an A4α, LYS–D-aspartic acid (ASP)-type murein, but the α-carboxyl group of the D-glutamic acid of the peptide subunit was substituted by a GLY residue. The analysis of the G+C contents of strains DMMZ 445, DMMZ 483, and DMMZ 537 revealed a range of 60 to 62 mol%; the G+C content of strain CUL 1808 was found to be 69 mol%.

In order to determine the precise phylogenetic positions of strains DMMZ 445, DMMZ 483, DMMZ 537, DMMZ 1369, and CUL 1808, PCR-16S rRNA gene sequence analyses were performed. A comparative analysis of the newly determined sequences with each other and with the sequences of other actinomycetes with high G+C contents retrieved from the EMBL Data Library was conducted. All five strains exhibited the highest sequence relatedness with representatives of the coryneform group of bacteria, specifically, species of the genera *Arthrobacter* and *Micrococcus* (Table 5). Strain DMMZ 1369 differed markedly from the other clinical strains examined but showed a 99.9% sequence similarity with *A. oxydans*. Strains DMMZ 445, DMMZ 483, and DMMZ 537 were found

TABLE 3. Antimicrobial susceptibility patterns of the strains studied

Strain	MICs (μg/ml) of the following antimicrobial agents[a]:															
	AMC	AM	CRO	CXM	CE	C	CIP	CLI	ERY	GM	IPM	PE	RIF	TEC	TET	VA
A. globiformis	0.5	0.5	8	4	1	4	4	1	0.25	1	1	0.25	≤0.03	0.06	0.25	0.25
A. atrocyaneus	2	1	16	4	2	8	8	2	0.13	4	4	1	0.06	0.5	0.5	0.5
A. aurescens	0.5	0.5	8	4	2	4	8	4	0.25	16	4	0.25	≤0.03	0.13	0.25	0.25
A. crystallopoietes	0.06	0.06	1	4	2	4	8	4	0.25	4	1	0.25	≤0.03	0.25	0.5	0.13
A. histidinolovorans	0.5	0.5	16	4	2	4	32	4	0.25	32	4	0.5	1	0.13	0.5	0.5
A. nicotinovorans[b]	S[c]	S	I[d]	I	S	S	R[e]	S	S	S	S	S	R	S	S	S
A. oxydans	1	0.5	16	8	2	4	4	2	0.25	8	4	0.25	≤0.03	0.13	0.25	0.13
A. pascens	0.5	0.5	8	4	1	2	4	1	0.25	2	2	0.5	≤0.03	0.06	0.25	0.13
A. ramosus	1	0.5	16	8	2	2	4	2	0.25	4	4	0.5	≤0.03	0.25	0.25	0.25
A. ureafaciens	0.5	0.25	8	4	1	4	32	4	0.25	32	4	0.5	2	0.25	0.5	0.25
A. nicotianae	0.5	0.25	8	4	1	4	4	4	0.5	1	1	0.13	0.25	0.06	0.5	0.06
A. protophormiae	2	1	8	2	1	2	4	2	0.25	2	4	0.5	≤0.03	0.06	0.25	0.13
A. uratoxydans	1	0.5	1	2	0.5	4	8	8	0.25	4	4	0.5	2	0.13	0.5	0.25
A. cumminsii DMMZ 445	≤0.03	≤0.03	0.06	≤0.03	≤0.03	2	1	0.25	0.13	4	0.13	≤0.03	≤0.03	0.13	0.25	0.25
A. cumminsii DMMZ 483	≤0.03	≤0.03	≤0.03	0.25	0.25	8	1	2	0.13	4	0.06	0.06	≤0.03	0.13	16	0.25
A. cumminsii DMMZ 537	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	8	2	0.13	0.13	2	0.06	≤0.03	≤0.03	0.13	16	0.25
A. woluwensis CUL 1808	8	8	>64	>64	32	8	16	4	2	32	8	4	32	0.06	2	2
Arthrobacter sp. strain DMMZ 1369	0.5	0.5	8	8	0.5	2	2	4	0.25	8	2	0.25	≤0.03	0.06	1	0.13
Arthrobacter sp. strain LCDC 90-0364	0.5	0.25	8	4	0.5	1	2	2	0.06	1	0.5	0.13	≤0.03	0.06	2	0.13
Arthrobacter sp. strain LCDC 91-0435	0.13	0.06	8	2	1	1	1	0.5	≤0.03	1	0.25	0.13	≤0.03	0.13	0.25	0.13
Arthrobacter sp. strain LCDC 92-0385	0.5	0.5	16	8	1	2	4	2	0.06	2	2	0.25	≤0.03	0.13	0.5	0.25
Arthrobacter sp. strain LCDC 92-0394	0.5	0.25	4	8	1	2	4	1	0.06	1	1	0.25	≤0.03	0.06	1	0.06
Arthrobacter sp. strain LCDC 92-0600	0.13	0.06	4	2	0.25	2	2	1	0.06	1	1	0.06	≤0.03	0.06	0.5	0.06
Arthrobacter sp. strain LCDC 93-0702	1	1	16	8	1	16	4	8	2	16	2	0.5	0.06	0.13	1	0.13

[a] AMC, amoxicillin-clavulanic acid; AM, ampicillin; CRO, ceftriaxone; CXM, cefuroxime; CE, cefalothin; C, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GM, gentamicin; IPM, imipenem; PE, penicillin G; RIF, rifampin; TEC, teichoplanin; TET, tetracycline; VA, vancomycin.

[b] The antimicrobial susceptibility pattern of *A. nicotinovorans* was determined by the disk diffusion method (19) on Mueller-Hinton agar plates supplemented with 5% sheep blood. Because NCCLS has yet not recommended breakpoints for disk diffusion testing of coryneform bacteria, the susceptibility test results for *A. nicotinovorans* should be considered presumptive.

[c] S, susceptible.

[d] I, intermediate.

[e] R, resistant.

to be genealogically closely related to each other (99.7 to 100% sequence similarity) but distinct from the other clinical strains and all reference *Arthrobacter* species. Strain CUL 1808 was genealogically distinct from all of the organisms included in the study. A tree depicting the phylogenetic positions of strains DMMZ 445, DMMZ 483, DMMZ 537, and CUL 1808 within the genus *Arthrobacter* is given in Fig. 2.

DISCUSSION

It is evident from the phenotypic and molecular genetic data that the three strains DMMZ 445, DMMZ 483, and DMMZ 537 and the single strain CUL 1808 are representatives of two new *Arthrobacter* species for which the designations *Arthrobacter cumminsii* sp. nov. and *Arthrobacter woluwensis* sp. nov., respectively, are proposed (see below). The three *A. cumminsii* strains (DMMZ 445, DMMZ 483, and DMMZ 537) exhibited at least 99.7% 16S rRNA sequence similarity with each other and displayed generally >4% sequence divergence from known reference *Arthrobacter* species, which is clearly indicative of a separate species (25). The results of the treeing analysis (Fig. 2) confirmed that these three clinical isolates represent a new and distinct subline within the genus *Arthrobacter*. Similarly, strain CUL 1808 was found to be genotypically distinct from all defined *Arthrobacter* and *Micrococcus* spp. The closest relatives of strain CUL 1808 are *Arthrobacter globiformis*, *Arthrobacter pascens*, and *Arthrobacter ramosus*. The levels of sequence divergence (ca. 2.5%) shown between CUL 1808 and these species is, however, significantly greater than that shown between some genomically distinct *Arthrobacter* species. This consideration, taken together with the isolated position occupied by strain CUL 1808 in the treeing analysis, demonstrates that this strain also represents a new *Arthrobacter* species.

Currently, it is exceedingly difficult to reliably determine the species of arthrobacters by conventional phenotypic (e.g., biochemical) tests. A chemotaxonomic marker, however, which has been shown to be particularly useful in the determination of arthrobacter species is the murein structure. Keddie et al. (13) gave a synopsis of the different murein types found in 15 *Arthrobacter* species [LYS-ALA$_{1-4}$, LYS-ALA-threonine (THR)-ALA, LYS-SER-ALA$_{2-3}$, LYS-SER-THR-ALA, LYS-THR-ALA$_2$, LYS-ALA-GLU, and LYS-GLU]; Kodama et al. (15) showed that the peptidoglycan of *Arthrobacter nicotinovorans* is type LYS-ALA-THR-ALA, and Koch et al. (14) showed that that of *A. agilis* is type LYS-THR-ALA. To confirm that *A. cumminsii* and *A. woluwensis* are distinct species, we elucidated the primary structures of the peptidoglycans of representative strains of each of these species (Fig. 1). The murein type of *A. cumminsii* is not found in any described *Arthrobacter* species. A similar murein type is known only for *Jonesia denitrificans*, with the exception that no GLY is detected. The murein of *A. woluwensis* CUL 1808 (Fig. 1) is, to our knowledge, unique. An additional peculiarity of this organism is that the D-alanyl–D-alanyl termini of peptide subunits not involved in cross-linkage are preserved because of lack of D,D-carboxypeptidase action. This lack is very exceptional and has been described only in *Lactobacillus gasseri* and *Lactobacillus johnsonii*. Thus, the presence of unique murein primary structures in these strains further demonstrates that *A. cum-*

TABLE 4. CFA patterns of the strains studied

Strain	% of total CFAs[a]												
	14:0*i*	14:0	15:0*i*	15:0*ai*	15:0	16:1*i*	16:0*i*	16:1ω7*c*	16:0	17:0*i*	17:0*ai*	18:1ω9*c*	18:0
A. globiformis	2	1	21	48			7	1	4	2	7	1	1
A. atrocyaneus	1	1	14	48			6		4	3	21	1	1
A. aurescens	2	1	4	73			3		3		12	1	1
A. crystallopoietes	1	1	3	75			4	1	4		7	2	2
A. histidinolovorans			4	59			4		3	1	25	1	1
A. nicotinovorans	2	1	6	62			10		3	1	14		1
A. oxydans	1	1	10	55			6	2	6	1	13	1	1
A. pascens		1	6	59		1	3	3	3	1	13	2	1
A. ramosus	1	1	6	63		2	3	3	2	1	8	1	
A. ureafaciens	4		8	56			15		3	1	11	1	1
A. nicotianae	2	1	9	50			13		5	2	15		
A. protophormiae	1	2	29	41			4		10	3	6	1	1
A. uratoxydans	1		27	34			7		4	11	14		1
A. cumminsii DMMZ 445	4	2	3	43			11		11		15	2	3
A. cumminsii DMMZ 483	4	3	3	48			9		10		11	3	4
A. cumminsii DMMZ 537	4	3	3	48			9		10		11	3	4
A. woluwensis CUL 1808	1		22	38			11		4	6	15	1	1
Arthrobacter sp. strain DMMZ 1369	1	2	12	54		1	3	6	3	1	8	2	1
Arthrobacter sp. strain LCDC 90-0364	3	2	6	46		1	12	3	7	1	12	2	2
Arthrobacter sp. strain LCDC 91-0435	1	2	7	46	1	1	6	7	6	1	13	1	1
Arthrobacter sp. strain LCDC 92-0385	2	1	8	56			8	1	6	1	11	1	2
Arthrobacter sp. strain LCDC 92-0394	4	1	5	52		2	13	2	5	1	10	2	2
Arthrobacter sp. strain LCDC 92-0600	3	1	7	48		3	11	4	5	1	10	2	2
Arthrobacter sp. strain LCDC 93-0702		2	6	55	1	4	4	6	7	1	13	1	

[a] Values of less than 1% are not reported.

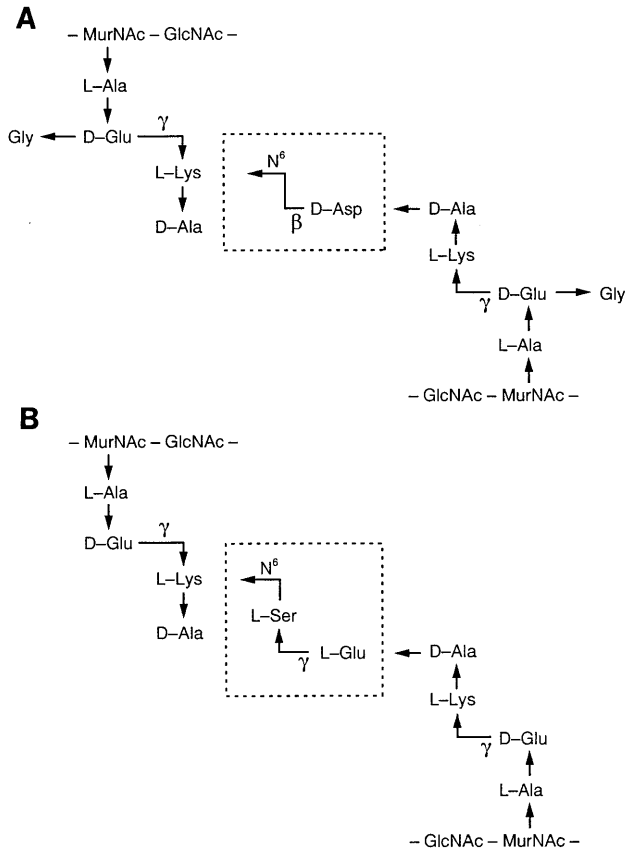

FIG. 1. Fragments of the peptidoglycan structures of *A. woluwensis* CUL 1808^T-type L-lysine–D-aspartic acid (A) and of *A. cumminsii* DMMZ 445^T-type L-lysine–L-serine–L-glutamic acid (B).

minsii and *A. woluwensis* represent two hitherto unknown *Arthrobacter* species.

The phenotypic identification of *Arthrobacter* spp. is made particularly difficult by the fact that the description of almost every defined *Arthrobacter* species is based on a single strain (13). Therefore, it might be necessary at present to supplement the identification of *Arthrobacter* spp. by peptidoglycan and/or molecular genetic methods, although we acknowledge that these methods are reserved for the reference laboratories.

It is emphasized that members of the genus *Brevibacterium* may be readily confused with *Arthrobacter* species. Strains of both genera show a marked rod-coccus cycle when grown on suitable media (11, 13). However, in Gram stains *Arthrobacter* strains may partially present as jointed rods which are not seen in true *Brevibacterium* strains. In our experience, jointed rods are rather rarely seen in clinical *Arthrobacter* isolates cultured on SBA. *Arthrobacter* strains may be motile, whereas *Brevibacterium* strains are invariably nonmotile. Many *Brevibacterium* strains give off a typical cheese-like odor (5) which is not noted for *Arthrobacter* strains. Analysis of CFA reveals that $C_{15:0ai}$ and $C_{17:0ai}$ predominate in *Brevibacterium* spp. (5), whereas only $C_{15:0ai}$ is the predominant CFA in *Arthrobacter* strains. Bernard et al. (1) reported the separation of CDC coryneform group B-1 bacteria into two different clusters on the basis of CFA analysis, from which it now seems likely that some so-called CDC coryneform group B-1b strains correspond to true *Arthrobacter* strains. Species determination within the genus *Arthrobacter* by means of CFA analysis was, in our hands, impossible, which is in line with the observations of Koch et al. (14). It is important that *meso*-diaminopimelic acid is the diamino acid in the cell walls of *Brevibacterium* strains (11), whereas lysine is found in *Arthrobacter* strains (13). The close phenotypic relationship between *Brevibacterium* spp. and *Arthrobacter* spp. is also indicated by previous confusion in the taxonomic placement of some species (e.g., *Brevibacterium pro-*

TABLE 5. Representative 16S rRNA sequence similarities of clinical and reference *Arthrobacter* and *Micrococcus* strains

Species	% Similarity																								
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	
1. *Arthrobacter* sp. strain DMMZ 445																									
2. *Arthrobacter* sp. strain DMMZ 483	99.7																								
3. *Arthrobacter* sp. strain DMMZ 537	99.7	100																							
4. *Arthrobacter* sp. strain DMMZ 1369	95.0	95.0	95.0																						
5. *Arthrobacter* sp. strain CUL 1808	95.2	94.9	94.9	95.5																					
6. *Arthrobacter atrocyaneus*	95.5	95.2	95.2	93.9	94.4																				
7. *Arthrobacter aurescens*	94.5	94.3	94.3	97.3	96.0	94.2																			
8. *Arthrobacter citreus*	94.4	94.3	94.3	95.7	93.9	93.6	96.1																		
9. *Arthrobacter crystallopoietes*	96.2	95.9	95.9	96.0	96.8	94.9	95.6	96.6																	
10. *Arthrobacter globiformis*	95.6	95.7	95.7	97.6	97.3	94.6	95.6	95.5	97.8																
11. *Arthrobacter histidinolovorans*	94.9	94.7	94.7	97.8	95.7	94.5	98.3	96.1	95.9	96.5															
12. *Arthrobacter ilicis*	94.3	94.1	94.1	96.1	95.4	93.9	98.6	95.0	95.0	95.1	98.2														
13. *Arthrobacter nicotianae*	95.7	95.5	95.5	95.9	96.5	94.7	94.3	93.3	95.8	96.6	94.8	93.9													
14. *Arthrobacter nicotinovorans*	94.9	94.9	94.9	98.0	95.9	94.6	98.3	96.7	96.2	96.8	99.6	98.3	95.0												
15. *Arthrobacter oxydans*	94.8	94.9	94.9	99.9	96.0	93.9	96.7	95.7	96.1	97.5	97.8	96.6	96.0	98.0											
16. *Arthrobacter pascens*	95.3	95.2	95.2	97.7	97.5	94.8	96.3	95.0	97.3	99.4	97.2	95.8	97.1	97.4	97.5										
17. *Arthrobacter polychromogenes*	94.8	94.9	94.9	99.9	95.8	93.7	96.7	95.7	95.8	97.5	97.7	96.5	95.7	98.0	99.9	97.5									
18. *Arthrobacter protophormiae*	95.5	95.2	95.2	95.2	96.9	94.1	95.3	93.2	95.5	96.2	94.6	94.7	98.4	94.9	95.3	96.7	95.1								
19. *Arthrobacter ramosus*	95.3	95.2	95.2	97.7	97.5	94.8	96.3	95.0	97.3	99.4	97.2	95.8	97.1	97.4	97.5	100	97.5	96.7							
20. *Arthrobacter sulfureus*	94.2	93.9	93.9	95.7	95.4	93.1	94.3	95.0	96.2	96.8	94.6	93.4	96.6	94.7	95.4	96.5	95.4	96.5	96.5						
21. *Arthrobacter uratoxydans*	95.0	94.8	94.8	95.3	95.7	93.6	93.7	93.4	94.9	95.7	98.6	92.9	98.1	94.1	95.2	95.7	95.2	97.0	95.7	96.0					
22. *Arthrobacter ureafaciens*	95.1	95.0	95.0	97.5	94.7	94.7	97.5	96.1	96.6	97.0	98.6	96.8	94.1	98.8	97.4	96.6	97.4	94.1	96.6	94.7	93.6				
23. *Arthrobacter agilis*	94.7	94.5	94.5	95.0	95.3	94.1	96.6	94.7	95.7	95.7	96.0	96.5	94.3	96.2	95.2	96.0	95.2	95.2	96.0	93.4	93.4	94.9			
24. *Micrococcus luteus*	95.2	94.9	94.9	95.7	96.0	94.9	94.9	94.1	95.7	96.0	95.1	94.6	96.4	95.4	95.5	96.6	95.6	96.0	96.6	95.7	95.5	94.5	94.7		
25. *Micrococcus lylae*	95.0	94.9	94.9	95.5	95.4	94.2	94.9	93.9	95.5	95.7	94.9	94.1	95.7	95.1	95.4	96.2	95.4	95.4	96.2	95.0	94.8	93.8	94.0	93.4	

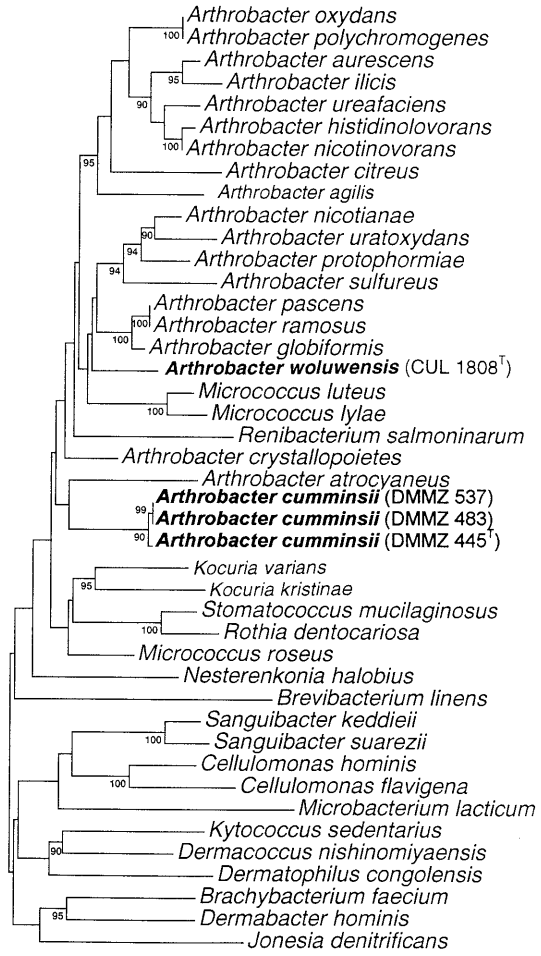

FIG. 2. Unrooted tree based on 16S rRNA gene sequences showing the phylogenetic position of *A. cumminsii* DMMZ 445^T and *A. woluwensis* CUL 1808^T within the genus *Arthrobacter* and close relatives. Significant bootstrap values (≥80) are indicated at the branching points.

tophormiae for *Arthrobacter protophormiae* and *Brevibacterium sulfureum* for *Arthrobacter sulfureus* [23, 24]).

Most likely, the overall pathogenic potential of *Arthrobacter* spp. is rather low because many people are presumably exposed to these bacteria. The mode of transmission of the isolates studied here is not clear, and to our knowledge, *Arthrobacter* spp. have not been described as inhabitants of human skin or mucous membranes. However, we believe that *Arthrobacter* strains would be more frequently reported if species or genus determination of clinically significant coryneform bacteria were performed more regularly. Clinical microbiologists should be aware of the possibility that previous CDC coryneform group B-1 and B-3 bacteria may not only represent *Brevibacterium* spp. (8) but may also represent *Arthrobacter* spp.

On the basis of the phenotypic and molecular genetic data presented here, we formally propose two new *Arthrobacter* species, *Arthrobacter cumminsii* sp. nov. for strains DMMZ 445, DMMZ 483, and DMMZ 537 and *Arthrobacter woluwensis* sp. nov. for strain CUL 1808, respectively.

***Arthrobacter cumminsii* sp. nov.** *Arthrobacter cumminsii* (cum′min.sii N.L. gen. n. *cumminsii*, of Cummins, to honor Cecil S. Cummins, a prominent American microbiologist and a pioneer of chemotaxonomy). The cells are coryneform bacteria without irregular branching. No spores formed. The cells are nonmotile. The organism is obligately aerobic. The colonies are whitish-grayish, smooth, slightly convex, and up to 2 mm in diameter after 24 h of incubation at 37°C in 5% CO_2 on SBA. The organism is catalase positive and nitrate reductase negative. Urea and esculin are not hydrolyzed. DNase and gelatinase activities are observed within 10 days. The organism does not utilize carbohydrates in the system described by Funke and Carlotti (5). The following enzyme activities are detected: esterase (C_4), esterase lipase (C_8), leucine arylamidase, cystine arylamidase, acid phosphatase, and phosphoamidase. Chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-glucosaminidase, α-mannosidase, and α-fucosidase are not present. The peptidoglycan type is L-lysine–L-serine–L-glutamic acid. The DNA base composition ranges from 60 to 62 mol% G+C. The organism has been isolated from human clinical specimens. The type strain (DMMZ 445) has been deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 10493. The type strain has the characteristics described for the species, and its G+C content is 60 mol%.

***Arthrobacter woluwensis* sp. nov.** *Arthrobacter woluwensis* (wo.lu′wen.sis N.L. fem. adj. *woluwensis*, from Woluwe, a town near Brussels, Belgium, where the type strain was isolated from a patient). The cells are coryneform bacteria which develop into cocci when the cultures become older (72 h). Jointed rods are observed after 1 to 2 days of incubation. No spores formed. The cells are nonmotile. The organism is obligately aerobic. The colonies are whitish-grayish, smooth, convex, and larger than 2 mm in diameter after 24 h of incubation at 37°C in 5% CO_2 on SBA. The organism is catalase positive. Nitrate is not reduced. Urea (72 h) and esculin (24 h) are hydrolyzed. DNase and gelatinase activities are detected within 24 h. Glycerol, galactose, D-glucose, D-fructose, D-mannose, dulcitol, mannitol, sorbitol, *N*-acetyl-glucosamine, amygdaline, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, D-raffinose, β-gentiobiose, D-turanose, D-arabitol, gluconate, 2-keto-gluconate, and 5-keto-gluconate are utilized. Erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, β-methyl-xyloside, L-sorbose, rhamnose, inositol, α-methyl-D-mannoside, α-methyl-D-glucoside, inulin, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, and L-arabitol are not utilized. The following enzyme activities are detected: alkaline and acid phosphatase, esterase (C_4), esterase lipase (C_8), lipase (C_{14}), leucine arylamidase, cystine arylamidase, trypsin, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. Chymotrypsin, α-galactosidase, β-glucuronidase, and α-fucosidase are not present. The peptidoglycan type is L-lysine–D-aspartic acid. The DNA base composition is 69 mol% G+C. The organism was isolated from cultures of human blood. The type strain (CUL 1808) has been deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 10495.

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